

WHAT IS CLAIMED IS:

1. A method of identifying one or more sequences of a target nucleic acid comprising:
 - a. contacting a target nucleic acid with a first set of pools of probes,
5 wherein at least one pool in the set comprises a mixture of two or more probes having different sequences in information regions of the probes, under conditions which produce, on average, more probe:target hybridization with probes which are perfectly complementary to the target nucleic acid in the information region of the probes than with probes which are mismatched in the information regions;
 - 10 b. detecting a first subset of pools for which a level of hybridization indicates that there is at least one perfectly complementary probe within each pool; and
 - c. identifying one or more sequences of the target nucleic acid from the first subset of pools detected in step (b) by compiling overlapping sequences of the information regions of the probes in the subset of detected pools, wherein one or more
15 pooling false positive probes are eliminated as a result of compilation of overlapping sequences.
2. A method of identifying one or more sequences of a target nucleic acid comprising:
 - 20 a. contacting a target nucleic acid with a first set of pools of probes, wherein at least one pool in the set comprises a mixture of two or more probes having different sequences in information regions of the probes, under conditions which produce, on average, more probe:target hybridization with probes which are perfectly complementary to the target nucleic acid in the information region of the probes than with
25 probes which are mismatched in the information regions;
 - b. assigning a hybridization score to each probe wherein each probe within a pool is assigned the same hybridization score, and
 - c. identifying one or more sequences of the target nucleic acid by analysis of hybridization scores of overlapping probes, wherein one or more probes with
30 false high scores arising from pooling of probes are eliminated by analysis of hybridization scores of overlapping probes.

3. The method of claim 2 wherein a statistical analysis of hybridization is performed in step (c).

4. The method of claim 3 wherein step (c) further comprises
5 calculating a score for the identified one or more sequences of the target nucleic acid.

5. The method of claim 1 further comprising, following step (b) and before step (c), the steps of:

10 a. contacting the target nucleic acid with a second set of pools of probes containing at least one probe having the same information region as a probe in the first set.

b. detecting a second subset of pools for which the level of hybridization indicates that there is at least one perfectly complementary probe within each pool; and

15 c. eliminating probes with the same information regions present in both the first set of pools of probes and the second set of pools of probes that are not present in both the first detected subset of pools and the second detected subset of pools.

6. The method of claim 5 wherein the first and second sets of pools
20 of probes comprise the same information regions.

7. The method of claim 5 wherein the first and second sets of pools of probes comprise the same probes.

25 8. The method of claim 2 further comprising, after step (b) and before
step (c), the steps of:

a. contacting the target nucleic acid with a second set of pools of probes containing at least one probe having the same information region as a probe in the first set,

30 b. assigning a hybridization score to each probe wherein each probe
within a pool is assigned the same hybridization score.

9. The method of claim 8 further comprising the step of:

c. eliminating the higher of two scores for probes present in both the first set and second set of pools of probes.

10. The method of claim 8 wherein the first and second sets of pools
5 of probes comprise the same information regions.

11. The method of claim 8 wherein the first and second sets of pools
of probes comprise the same probes.

10 12. The method of claim 1 or 2 in which the target nucleic acid is
labeled.

13. The method of claim 1 or 2 in which the probes are labeled.

15 14. The method of claim 1 or 2 in which the label is a fluorophore.

15 15. The method of claim 1 or 2 in which the label is attached to a
terminal nucleotide.

20 16. The method of claim 1 or 2 in which the label is attached to an
internal nucleotide.

25 17. The method of claim 1 or 2 in which the first set of pools of probes
is immobilized on one or more solid supports.

18. The method of claim 17 in which the pools of probes are arranged
in a spatially-addressable array in which each pool has a unique address.

30 19. The method of claim 1 or 2 in which the target nucleic acid is
immobilized on one or more solid supports.

20. A method of identifying one or more sequences of a target nucleic
acid comprising:

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5 a. contacting a target nucleic acid with a first set of pools of immobilized probes and a first set of pools of labeled probes, wherein at least one pool in either the first set of pools of immobilized probes, or in the first set of pools of labeled probes, or in both, comprises a mixture of two or more probes having different sequences in the information regions of the probes, under conditions which produce, on average, more probe:target hybridization for probes which are perfectly complementary to the target nucleic acid in the information region than with probes which are mismatched in the information region;

10 b. covalently joining adjacently hybridized immobilized probes and labeled probes to provide a first set of covalently joined probes;

c. detecting a first subset of pools of covalently joined probes for which a level of hybridization indicates that there is at least one perfectly complementary covalently joined probe within each pool; and

15 d. identifying one or more sequences of the target nucleic acid from the first subset of covalently joined pools of probes detected in step (c) by compiling overlapping sequences of the information regions of covalently joined probes in the subset of detected pools, wherein one or more covalently joined pooling false positive probes are eliminated as a result of compilation of overlapping sequences.

20 21. The method of claim 20 further comprising, following step (c) and before step (d), the steps of:

25 a. contacting the target nucleic acid with a second set of pools of immobilized probes and a second set of pools of labeled probes, wherein at least one probe in the second set of immobilized probes has the same information region as a probe in the first set of pools of immobilized probes, or at least one probe in the second set of labeled probes has the same information region as a probe in the first set of pools of labeled probes,

b. covalently joining adjacently hybridized immobilized probes and labeled probes to provide a second set of covalently joined probes;

30 c. detecting a second subset of covalently joined pools of probes for which a level of hybridization indicates that there is at least one perfectly complementary probe within each pool; and

- d. eliminating covalently joined probes with the same information regions present in both the first set of covalently joined pools of probes and the second set of covalently joined pools of probes that are not present in both the first detected subset of covalently joined pools of probes and the second detected subset of covalently joined pools of probes.

22. A method of identifying one or more sequences of a target nucleic acid comprising:

- a. contacting a target nucleic acid with a first set of pools of immobilized probes and a first set of pools of labeled probes, wherein at least one pool in either the first set of pools of immobilized probes, or in the first set of pools of labeled probes, or in both, comprises a mixture of two or more probes having different sequences in the information regions of the probes, under conditions which produce, on average, more probe:target hybridization for probes which are perfectly complementary to the target nucleic acid in the information region than with probes which are mismatched in the information region;
- b. covalently joining adjacently hybridized immobilized probes and labeled probes to provide a first set of covalently joined probes;
- c. assigning a hybridization score to each covalently joined probe in the first set wherein each probe within a pool of covalently joined probes is assigned the same hybridization score, and
- e. identifying one or more sequences of the target nucleic acid from overlapping covalently joined probes by analysis of hybridization scores of overlapping covalently joined probes wherein one or more covalently joined probes with false high scores arising from pooling of probes are eliminated by analysis of hybridization scores of overlapping probes.

23. The method of claim 22 further comprising after step (c) and before step (d) the steps of:

- a. contacting the target nucleic acid with a second set of pools of immobilized probes and a second set of pools of labeled probes, wherein at least one probe in the second set of immobilized probes has the same information region as a probe in the first set of pools of immobilized probes, or at least one probe in the second set of

labeled probes has the same information region as a probe in the first set of pools of labeled probes,

b. covalently joining adjacently hybridized immobilized probes and labeled probes to provide a second set of covalently joined probes;

5 c. assigning a hybridization score to each covalently joined probe of the second set wherein each probe within a pool of covalently joined probes is assigned the same hybridization score.

24. The method of claim 23 further comprising the step of

10 d. eliminating the higher of two scores for covalently joined probes present in both the first set and second set of covalently joined pools of probes.

25. The method of claim 21, 23 or 24 wherein the first and second sets of pools of immobilized probes, or the first and second sets of pools of labeled probes, or
15 both, comprise the same information regions.

26. The method of claim 21, 23 or 24 wherein the first and second sets of pools of immobilized probes, or the first and second sets of pools of labeled probes, or both, comprise the same probes.
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27. The method of any one of claims 20 through 24 in which a label of the labeled probe is a fluorophore.

28. The method of any one of claims 20 through 24 in which a label of
25 the labeled probe is attached to a terminal nucleotide.

29. The method of any one of claims 20 through 24 in which a label of the labeled probe is attached to an internal nucleotide.

30. The method of any one of claims 20 through 24 in which the set
30 of pools of immobilized probes is immobilized on one or more solid supports.

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31. The method of claim 30 in which the sets of pools of immobilized probes are arranged in a spatially-addressable array in which each pool has a unique address.

5 32. The method of claim 22, 23 or 24 wherein a statistical analysis of hybridization scores is performed.

33. The method of claim 22 wherein step (d) further comprises calculating a score for the identified one or more sequences of the target nucleic acid.

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34. The method of any one of claims 20 through 24 wherein the pools of immobilized probes each consist of one probe.

35. The method of any one of claims 20 through 24 wherein the pools of labeled probes each consist of one probe.

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36. A set of pools of probes wherein each probe comprises an information region, wherein said set of probes is sufficient to determine the sequence of an unknown target nucleic acid by overlapping sequences of the information region of two or more of said probes, and wherein at least one pool comprises two or more probes having different sequences in the information regions and having the same label or no label, and wherein the set of the pools of probes also satisfies one or more of the following rules describing the information regions of the probes, said rules selected from the group consisting of:

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25 a. a consensus sequence of at least one pool in the set consists only of the letters selected from the group consisting of V, H, D, B, and N;

b. a consensus sequence of probes in each pool in the set comprises more than three different letters selected from the group consisting of A, C, G, T, U, M, R, W, S, Y, K, V, H, D, B, and N;

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c. consensus sequences from each informative position of all pools in the set comprise more than eight letters selected from the group

consisting of A, C, G, T, U, M, R, W, S, Y, K, V, H, D, B, and N;
and

- d. consensus sequences from each information region of all pools in the set comprise more than five different letters selected from the group consisting of A, C, G, T, U, M, R, W, S, Y, K, V, H, D, B, and N, wherein at least one letter is selected from the group consisting of M, R, W, S, Y, and K.

37. The set of pools of probes of claim 36 wherein said set comprises
10 all possible probes of the same length K, where K is greater than 3.

38. The set of pools of probes of claim 36 wherein each pool comprises more than 16 different probes.

15 39. The set of pools of probes of claim 38 wherein each pool comprises
at least 32 different probes.

40. The set of pools of probes of claim 36 in which the pools are arranged in a spatially-addressable array, and wherein each pool has an address.

41. The set of pools of probes of claim 36 wherein at least two pools are mixed, wherein any two pools that are mixed are associated with different labels, and wherein all probes in a single pool are associated with the same label.